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ABSTRACT

The purpose of the manual is to provide the medical assisting student a text which presents the common laboratory procedures in use today in physician's offices. The procedures for performing a complete urinalysis are outlined, along with those for carrying out various hematological tests. Information is also presented to help the student learn to identify forms of bacteria, to become acquainted with the various test media for growing cultures, and to learn to prepare smears and stains. A review test follows each of the three units of study. These procedures are based on the requirements for the certifying examination given by the American Association of Medical Assistants. The procedures are not intended to encompass the advanced skills needed in medical technology. (Author/NJ)

STATE OF NEW JERSEY
DEPARTMENT OF EDUCATION
DIVISION OF VOCATIONAL EDUCATION

LABORATORY PROCEDURES FOR MEDICAL ASSISTANTS

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Division of Vocational Education

LABORATORY PROCEDURES FOR MEDICAL ASSISTANTS

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MANUAL FOR MEDICAL ASSISTANTS
LABORATORY PROCEDURES

The purpose of this manual is to give the Medical Assisting student a text which presents the common laboratory procedures in use today in physicians' offices. Many textbooks emphasize the office skills required with nothing more than a mention of the laboratory tests. This gap in the field of assisting prompted the writing of this manual.

The procedures included are based on the requirements for the certifying examination given by the American Association of Medical Assistants. The ability to properly perform these tests demonstrates knowledge, skill and proficiency in one phase of Medical Assisting. The procedures are not intended to encompass the advanced skills needed in Medical Technology.

I. Urinalysis

Objectives:

Perform complete urinalysis for identification of existing pathological conditions.
Demonstrate ability to use the various test media in urinalysis procedures.

A. General physical characteristics:

The quantity of urine may vary from day to day in healthy people. Factors affecting this could be fluids consumed, amount of perspiration, etc. Average daily output is 1200 cc. to 1500cc. (40 to 50 oz.)

Daytime voiding produces larger quantities than night time voidings in the proportions of approximately 3.5:1. Quantities in excess of 3,000 cc. in 24 hours are produced by polyuria. Oliguria is decreased urinary output. Anuria is total urinary suppression.

1. Quantity: The actual amount of urine voided may be determined by pouring the specimen into a volumetric container and recording the amount.

2. Color: The color is determined by examination with the naked eye. The range of color is from straw colored through amber or reddish brown. Some other descriptive terms used are pale straw, moderate yellow and dark yellow. The color will depend on the quantity voided; dilute urine being pale and concentrated urine being dark. Alkaline urine will be darker than acid urine. Blood will give a red or brown, smoky color. Increased amount of blood is called hemoglobimuria. Bile will lend a yellow tinge that will turn to green upon standing. A pale green urine will usually indicate sugar diabetes.

3. Appearance: Urine will appear clear or cloudy in various degrees. If cloudy note it as slightly, moderately, or very cloudy. It also should be noted if there is precipitation in the bottom of the container. Usually freshly passed urine will appear clear but if left standing will develop a cloudy appearance caused by bacteria and alkaline salts due to decomposition. After standing, a cloud of mucus, leukocytes and epithelial cells settle to the bottom. This cloudiness, a nebecula, has no clinical significance. This substance is more readily found in the urine of women due to vaginal discharge and cells.

4. Specific Gravity is the weight of a specific volume of liquid as compared with the same volume of distilled water. The specific gravity of urine is a measure of kidney function. The inability of the kidneys to dilute urine to a specific gravity after taking in large measured amounts of water or the inability to concentrate it after fluid restriction indicates renal dysfunction. The normal range for specific gravity is 1.003 to 1.030. The normal urine specific gravity is 1.015 to 1.025. In disease, the range may vary from 1.001 to 1.060.

Test for Specific Gravity:

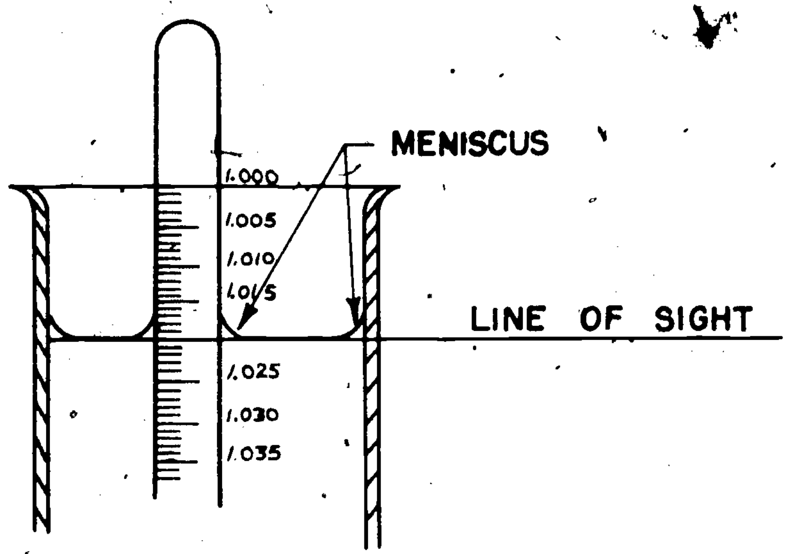
If the amount of urine is insufficient to perform the test it may be diluted by adding an equal amount of distilled water. In this case the last two figures of the reading must be multiplied by two for an accurate reading.

Materials

Squibb Urinometer or Midget (Junior) Urinometer
Sufficient urine to perform test (concentrated or diluted)
Volumetric container

1. Fill the cylinder ¾ full with well mixed urine – eliminate bubbles
2. Lower the float
   Do not let the float touch the sides or bottom of the cylinder.
3. The reading is taken from the bottom of the meniscus while holding the cylinder at eye level
4. Record the results as: 1.020 S/G

Note: If urine has been diluted do not use for qualitative or quantitative studies.



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5. Reaction: pH

Normal urine may be acid, alkaline or amphoteric at various times. Freshly voided urine is usually acid with an average reading of pH 6.0. The normal range is 4.8 through 7.5 with readings below 7.0 recorded as acid and those above as alkaline. Diet is the most important factor affecting the pH. Acidity is caused by ingesting large amounts of protein and alkalinity by the recent digestion of large meals, after abundant eating of fruits and administration of certain drugs such as sodium bicarbonate.

Materials
Nitrazene paper
Urine specimen

Procedure for pH
Dip a piece of Nitrozene paper into the specimen. After one minute compare the color of the test paper with the color chart.
Chart reads from pH 4.5 to 7.5

B. Method of collecting
Objectives:
Learn the importance of proper collecting methods
Become familiar with preservatives used

Most specimens will be collected in the office and for this purpose, a wide mouthed bottle is usually provided. There are numerous disposable containers on the market that are adequate for this purpose. If the specimen is to be collected at home, the patient should, preferably, be given a container from the office. If this is not possible instruct the patient to use a clean wide-mouthed jar. Be careful to stress the importance of cleanliness as any residue left in the container may invalidate the studies.

1. Preservatives:

Urinalysis should be performed as soon as possible after the specimen has been voided so that decomposition does not occur. If this is not possible, preservatives should be employed.

a. refrigeration is the preservative commonly employed if the delay is not too extended.
b. Formalin – one drop per ounce of urine
c. Thymol – this is a crystalline substance and may interfere with tests done for protein or bilirubin
d. Toluene – this is the most commonly used preservative. Enough Toluene is added to form a thin layer on the specimen. It must be pipeted off or skimmed off before examination.

REVIEW OF PHYSICAL CHARACTERISTICS OF URINE

A. Using a freshly voided specimen of urine record the following:

Urinalysis
Physical Examination

Name ______________________ Date ______________________

Amt. (24 hrs.) ______________________ Reaction pH ______________________

Color ______________________ Sp. Gravity ______________________

Appearance ______________________

B. Using Nitrazene paper test the pH of urine specimens and orange, apple and tomato juices.

C. Using a urinometer measure, record and compare the specific gravity of the following:

Water	Saline
Urine	Milk

Answer the following questions:

1. Name the factors influencing the output of urine.

____

2. Define the following:
   Polyuria
   Anuria
   Oliguria
3. When urine is left standing it will begin to ______________
4. What are the substances in the nebecula?
5. The normal range for specific gravity is ______________
6. Why is the specific gravity significant?
7. The normal pH range of urine is ______________
8. One cause of urine acidity is ______________
9. Alkalinity may be caused by ______________
10. The principle preservative for urine is ______________
11. Give one reason why it might be necessary to dilute urine used in the S/G test. ______
12. Briefly describe the procedure used for testing the pH of urine
13. Briefly describe the procedure for S/G.
14. Name the three chemical preservatives for urine and state their advantages and disadvantage

C. Chemical Examination

Objectives:

Acquire skill in performance of qualitative and quantitative chemical examinations

Recognize the normal and abnormal test results

Accurately read and record test results

1. Urine is a complex solution composed of organic and inorganic substances. Most of these substances are either waste products from metabolism or derived from ingested foods. Of the approximately 35 organic substances the most important are urea, uric acid and creatinine. Urea composes about half of the organic substances. The most important inorganic substances are chlorides, phosphates, sulfates and ammonia. Sodium Chloride makes up about half of the inorganic substances.

   Certain substances are present only when pathological conditions are present. Those most commonly found in pathology are proteins, carbohydrates, acetone, bile and blood.

   Chemical examinations of urine are usually qualitative in nature to determine if any abnormal substances are present. If qualitative tests are positive the physician will then order quantitative tests to determine the amount of pathogenic substances present.

   The most common of these tests and the ones to be explained here are those for glucose, acetone, albumin, blood and bile.

   a. Glucose

      Glycosuria is of little importance and is usually transient except in some cases where it may be the first indication of diabetes mellitus.

      (1) Reagent Strip (Qualitative test)

      The reagent strip will react to concentrations of glucose as low as .01 percent. It will not react to reducing substances or sugar other than glucose.

      Materials

      Test Strip

      Urine Specimen

      Procedure:

      (a) Dip the test strip briefly into the urine
      (b) Do not touch the end of the strip
      (c) Read the color ten seconds after dipping. No change indicates negative for glucose. If a shade of purple develops the test is positive and quantitative tests are indicated.

(2) Reagent Tablet (Quantitative)

Materials needed

water – a small amount	Reagent tablet
Test Tube	Medicine dropper

Procedure:

1. Hold the dropper in an upright position and place five drops of urine into an upright test tube. Rinse the dropper and place ten drops of water into the same tube. Place the test tube in the rack.
2. Drop one reagent tablet into the test tube. Watch the reaction carefully for the specified fifteen seconds. Do not touch or shake the test tube during this time.
3. After the waiting time shake the tube gently and compare with color chart.
4. Report as follows:

Negative	=	blue (any shade)	0.00%
Trace	=	dark green	0.25%
+	=	grass green	0.5%
++	=	green brown	0.75%
+++	=	tan	1.00%
++++	=	orange	2.00% or over

An amount of sugar over 2% causes rapid color changes to green, tan, orange and then to dark shade of greenish grown. Watch the reaction carefully for even a fleeting change through orange to greenish brown which will indicate the presence of over two percent sugar and should be reported as such regardless of the final color change.

(3) Benedict's Qualitative Test for Glucose

Materials

Benedict's Qualitative Solution	Urine specimen
Pyrex test-tube	Medicine dropper
Bunsen Burner	

Procedure:

Place ten drops of urine in a pyrex test tube. Add 5 cc. of solution and mix by shaking gently. Heat to boiling while gently shaking the tube. Be careful not to splatter the contents. Remove from heat and cool. Boil a second time and remove from heat. When bubbling stops the test may be read as follows:

Blue color	Negative	Deep yellow green ++
Green-blue	Trace	Yellow-tinge of green +++
Yellow-green	+	red yellow ++++

b. Acetone

Acetone is referred to as ketone bodies which are acetone-like substances. These substances result when there is a breakdown of body fat such as in dieting or in a diabetic when the utilization of glucose is impaired.

Materials
a Reagent strip
urine specimen

Procedure:

(1) Dip the strip into a freshly voided specimen of urine.
(2) Remove strip immediately and tap against the edge of the container to remove the excess.
(3) Wait fifteen seconds and compare the strip with the color chart on the container.
(4) Report as follows:
Negative: no color change
Positive: Intensity of the purple color dependent on the amount of ketones present

c. Reagent Tablet

Materials

Small piece of white paper	Reagent tablet
Medicine dropper	Urine sample

(1) Place the tablet on a clean piece of white paper.
(2) Place one drop of urine on the tablet
(3) Wait thirty seconds for reaction.
The drop should be absorbed in this time. If it is not absorbed discard the tablet as it has become moisture laden.
(4) Report results as follows:
Negative: cream colored or unchanged
Positive: Lavender – trace, purple – moderate, deep purple – strongly positive.

d. Proteins:

Serum albumin and serum globulin are the most important of the proteins found in the urine. Albuminuria is the term used to designate their presence in urine. Proteinuria is the most common sign of kidney disease and its presence serves as the best indicator of renal abnormality. Albuminuria may be either renal or accidental. Accidental or false albuminuria may occur in vaginal infections, as well as blood or pus mixing with the urine from other sites. Renal albuminuria occurs when albumin has passed from the blood into the urine through walls of the kidney tubules or the glomeruli.

Materials
Urine sample
Reagent strip

(1) Procedure: Reagent Strip Method
(a) Dip the yellow end of the strip briefly into the specimen
(b) Compare the color of the strip with the color chart provided. If the urine contains protein it will exhibit changes from a green through a blue. The color reaction will be dependent on the amount of protein present.

(2) Reagent Tablet Method

Materials
Urine sample
Reagent tablet
Small amount of water
Medicine dropper

Procedure:
(a) Place the tablet on a clean surface and put one drop of urine on the tablet
(b) After the urine has been absorbed add two drops of water and wait for absorption
(c) Compare the color that appears on the top of the tablet with the package illustration. The color varies from green to blue in the presence of protein.

e. Acetic Acid and Heat Test for Albumin

Materials
10% Acetic Acid
Centrifuge
Two test tubes – equal sizes

Procedure
Centrifuge or filter the urine to clearness. Pour equal amounts of urine into two test tubes. Be certain the test tubes are of the same diameter. One tube will be the control tube.

Heat the upper portion of the urine in one tube until it begins to boil. If a cloud appears it indicates the presence of albumin, phosphates or carbonates. The next step is to rule out all but the albumin. Add three or four drops of acetic acid to the tube and shake gently. If the cloud remains, heat the urine again and add a few more drops of the acid.

READ THE RESULTS AS FOLLOWS:
Compare the two test tubes. If there is no cloud the test is negative. If there is a slight cloud the reading is a trace of albumin. Easily discerned cloud is read as +, more-definite cloud as ++, dense cloud as +++ and thick cloud as ++++

f. **Blood**

In urine blood may occur as hematuria or hemoglobinuria.
Hematuria is the presence of more than the normal number of red cells. Hemoglobinuria is the hemoglobin from disintegrated red blood cells or their pigment. In testing for occult blood one must rule out the possibility of numerous red cells giving a false positive reading. This possibility can be eliminated by examining the sediment microscopically. If the sediment has no more than the normal number of red cells, the positive reading for occult blood is true.

(1) Benzidine Test for Occult Blood

Material

Benzidine Solution	Centrifuge
Hydrogen Peroxide	Medicine Dropper

Procedure:
Centrifuge a tube of urine and pour off the clear fluid. Retain the sediment and to it add a few drops of Benzidine Solution and an equal amount of peroxide.
Shake the solution to mix.

READ RESULTS AS FOLLOWS:
If sediment turns blue the test is positive for occult blood. The readings are from a trace–faint, greenish blue to ++++ a very deep blue.

(2) Reagent Strip Method

Materials
Reagent strip
Urine specimen

Procedure:
(a) Dip test portion of strip into fresh urine. Remove immediately.
(b) Tap gently against edge of container to remove excess.
(c) Wait thirty seconds to read. No color change indicates negative results. Green-blue color developing indicates positive results.

(3) Reagent-Tablet Method

This is a highly sensitive reagent tablet and will reveal hemolyzed and intact red blood cells giving it an advantage over microscopic studies. It is sensitive to the degree of one part blood to 100,000 parts urine.

Materials

Reagent tablet	Small amount water
Filter paper square	Medicine dropper

Procedure:

1. Place one drop of urine in the center of the filter paper square.
2. Put the reagent tablet in the center of the moist area.
3. Place two drops of water on the tablet. A small amount of water must spill onto the test area and this will occur when fizzing of the tablet takes place.
4. Negative: No blue area appears on FILTER PAPER at the end of two minutes

   Positive: The FILTER PAPER surrounding the tablet turns blue. The intensity, speed and amount of color determine the amount of blood present. Disregard color changes on the tablet or those after two minutes.

g. Bile

A test for bile in the urine serves several purposes. It may be the first indication of liver dysfunction or common bile duct obstruction. This test is helpful in following the course of infectious hepatitis. It is also used in screening prospective blood donors.

Materials

Reagent Tablet	Medicine Dropper
Test Mat	Small amount of water

Procedure

(1) Place five drops of urine on the special test mat supplied with the tablets.
(2) Place the tablet in the center of the moist area.
(3) Flow two drops of water on the tablet. Blue or purple color on the MAT around the tablet within 30 seconds indicates a positive reaction. Ignore any pink or red colors that result.

Pregnancy Test

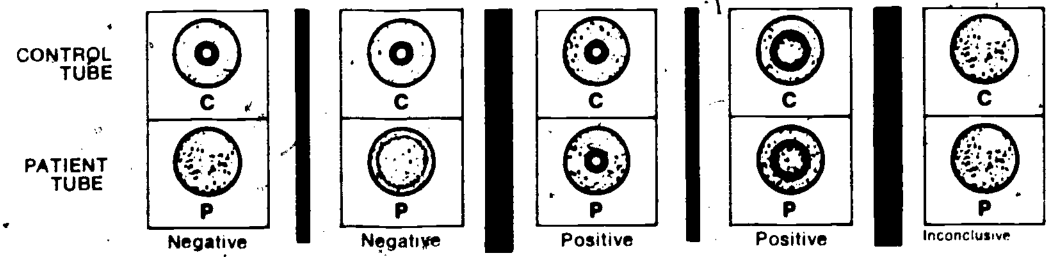
One of the simplest and most accurate UCG tests for pregnancy now in use is a two hour pregnancy test. It has an accuracy rating of 97 to 99% using urine and 96% using serum. All necessary supplies and directions are included.

D. UCG – Pregnancy Test

Materials
Distilled Water
Small funnel and filter paper or
Centrifuge
Tuberculin type syringes, pipets
or calibrated droppers for use with ¼ cc., .05mm.
Test Tubes – round bottom with outside diameters 9.5 – 10.0 mm. Wall thickness .60 ± Length 75 mm. ± 1 mm.

1. Procedures
Dilute one volume of clear urine with two volumes of distilled water.
Place two test tubes in the rack marked P (patient) and C (control)
Fill dropper to line from bottle marked Control and deliver to control tube
Fill dropper to line from bottle marked HCG – Antiserum and place contents in test tube marked patient
Place ¼ cc.of the diluted urine into each test tube.
Shake the bottle marked cell suspension and put one drop into each test tube.
Hold the tube between your thumb and index finger and with the other finger flick the bottom of the test tube until a homogenous mixture results.
Place the tubes in the racks.
Allow the test tubes to remain for two hours and then read.
For best results read through bottom of the tube.

Read as per illustrations



2. Sources of error
All materials must be clean and free of residue from prior tests.
Test tubes other than those recommended will result in invalidating the test results.
Test must remain undisturbed for two hours.
Attempting to read results at eye level rather than through bottom of tube may result in false reading.

3. Storage of reagents
The reagents must be refrigerated to preserve their life. Temperature should remain at between 2–8°C.

ACTIVITIES AND REVIEW

1. Using a urine specimen from a volunteer, test for the following: sugar, acetone, albumin, blood and bile.
2. Using the above results record your findings on this form.

URINALYSIS

Name ________________________ Date ________________________

Sugar
- Reagent strip ________________
- Reagent tablet ________________

Acetone
- Reagent strip ________________
- Reagent tablet ________________

Albumin
- Reagent strip ________________
- Reagent tablet ________________

Blood
- Reagent strip ________________
- Reagent tablet ________________

Bilirubin
- Reagent tablet ________________

3. What is the difference between qualitative and quantitative chemical analysis?
4. Name the two basic substances that compose urine.
5. Name five abnormal substances found in urine.
6. The presence of ____________ ____________ in the urine may be the first indication of diabetes mellitus.
7. Acetone is referred to as ____________ bodies.
8. ____________ and ____________ are the most important of the proteins found in the urine.
9. ____________________ is the most common sign of kidney disease.
10. Accidental or false albuminuria may occur in ____________
11. In urine blood may occur as either hematuria or ____________
12. Define the following:

glycosuria	hematuria
ketone bodies	albuminuria
serum albumin	serum globulin

13. A test for bile in the urine may be the first indication of ____________
14. Rings of approximately the same size and intensity in both the patient and control tubes of the UCG Pregnancy test would be reported as ____________

E. MICROSCOPIC EXAMINATION OF URINE

Objectives:

Learn how to examine urine microscopically. Identify normal and abnormal components of urine. Learn the classification of structures present in urine

1. Crystals: Crystals are the unorganized sediment found in urine. They may be classified according to the pH reaction of the urine in which they may be found.

   a. Crystals in acid urine

      (1) Uric Acid Crystals
      These are yellow or reddish brown crystals that form rosette-like crystals or rhombic plates. They are not considered significant unless found with blood cells in a freshly passed specimen.

      (2) Calcium Oxalate Crystals
      These crystals are significant only when found in clumps accompanied by renal irritation. They appear as colorless glistening square crystals crossed by two intersecting diagonal lines.

      (3) Amorphous Urates
      These are fine, yellowish, almost colorless granules common in concentrated and strongly acid urine. If they obscure other structures in examination, the urine may be warmed to dissolve them.

   b. Crystals Found in Normally Alkaline Urine

      (1) Triple Phosphate Crystals
      These are colorless and shaped like stars, rosettes and prisms. When rapidly deposited they assume a feather-like form.

      (2) Calcium Phosphate Crystals
      These take the form of jagged-edged crystals which are large, flat and colorless.

      (3) Ammonium Biurate
      This is the only urate deposited in alkaline urine. It forms opaque yellow crystals, usually in spheres, thorn apples and sheaves of needles. It is generally found with phosphates in decomposed urine and is of no significance.

   c. Abnormal Crystals Found in Acid Urine

      (1) Leucine – These are slightly yellow, oily looking spheres with radial striations.

      (2) Tyrosine – These crystals look like black sheaves of very fine needles constricted in the center.

(3) Cystine – These crystals are colorless, highly refractile rather thick hexagonal plates.

(4) Cholesterol – These appear as large plates similar to calcium phosphates crystals in appearance.

2. Casts – are structures formed in the renal tubules.
   These are rarely found in urine which does not or has not recently contained albumin. Their presence indicates a change in the kidney either slight or transitory. They should be searched for with a low power objective and a slightly darkened field.
   a. Hyaline Casts – these are colorless, semitransparent cylindrical structures with rounded ends. They are of little significance and may appear in some transitory conditions.
   b. Granular Casts – these are hyaline casts containing granules. They are designated as finely or coarsely granular casts, depending on size of the granules.
   c. Fatty Casts – these are casts in which small droplets of fat appear. If they are considerable in number they may indicate partial or complete degeneration of the renal epithelium.
   d. Waxy Casts – these casts are gray or colorless and have a waxy appearance. They occur in advanced nephritis and indicate serious pathology.
   e. Cylindroids – these casts resemble hyaline casts but have a long, tapering tail that is often twisted or curled.
   f. Mucous Threads – these are very faint, twisted strands. They usually appear in all urine in moderate amounts.
   g. Bacteria – appear as extremely tiny rods or spheres. They are of no significance unless the specimen has been obtained by catherization.
   h. Spermatozoa – their presence is usually as a contaminant if appearing in a woman's urine and need not be reported. If they occur in the urine of a male they should be reported to rule out the possibility of spermatorrhea.

3. Organized sediment
   Organized sediment refers to cells and other formed elements which may be from any part of the genito-urinary tract.
   The most important structures to be identified are tissue cells, casts and red and white blood cells. It is necessary to report other structures which might appear also.
   a. Epithelial cells
      Epithelial cells may appear in the urine of females just prior to or following menstruation. An increased amount indicates the presence of a pathological condition. The abnormal cells differ from the normal in that the nuclei may be obscured. They are round or oval granular cells that sometimes appear with an irregular periphery.

b. Leukocytes or Pus Corpuscles

A few leukocytes are usually present in normal urine in the amount of approximately 0–3 per high power field. If their numbers are increased this indicates pathology. In this case they are called pus corpuscles and their presence is called pyuria. Suppuration is occuring in some part of the urinary tract when these are found. They have a granular, strippled appearance which obscures the nucleus.

c. Erythrocytes

These are tiny, round or oval, slightly concave disks with no granulation and are usually one-third to one-half the size of leukocytes. They may be slightly swollen in alkaline urine and slightly crenated in very acid urine. If blood appears in the urine and it is not the result of contamination from menstrual discharge, it is indicative of a pathological condition.

4. Extraneous Structures Found in Urine

Certain substances are present as the result of accidental contamination of the urine. Recognition of these structures should be made so as to avoid error in reporting:

- Air bubbles
- Feathers
- Fibers
- Hairs
- Molds
- Muscle tissue
- Oil globules
- Scratches on glass slide or cover glass
- Starch granules
- Vegetalbe tissue
- Yeast cells

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MICROSCOPIC EXAMINATION OF URINE

All urine contains sediment and microscopic examination is part of every urinalysis. Since sediment decomposes if left standing, the examination should be carried out within six hours after the specimen has been voided. If this is not possible, a small amount of formalin should be added or the sample should be refrigerated.

Procedure

1. Spin a well mixed urine sample in the centrifuge for 3 minutes at 1,800 r.p.m.
2. Pour the supernatant fluid off and place a drop of the sediment on a clean glass slide. Cover the slide with a cover glass.
3. Place the slide on the microscope stage. Examine at least six different fields for casts with the low power field.
4. Switch to the high-power objective to differentiate between the various structures. The low power is used to locate while the high power is used to identify the structures.
5. After identification record the results. With some structures it is sufficient to note as occasional, few, numerous or too numerous to count. With others they must be estimated as 1–4 per HPF.

Sources of Error

1. careless transfer of sediment
2. use of too much light
3. use of high power only
4. drying of sediment
5. dirty equipment

MAGNIFICATION FOR PROPER REPORTING

Structures	Field Requirement
Casts*	per LPF*
W.B.C.	per HPF
Clumps (pus)	per HPF
R.B.C.	per HPF
Epithelial cells	per LPF
Crystals	per LPF
Amorphous Urates	per LPF
Cylindroids	per LPF
Bacteria**	per HPF
Mucous threads	per LPF

*Casts are large and LPF is acceptable

**Note if motile or nonmotile

Review

1. Practice performing a urinalysis and report the findings on the form below:

COMPLETE URINALYSIS

Patient ____

Date ________________ Age ________________ Sex ____________

Physical Examination

Color ____________________________ Transparency ______________

Specific Gravity ______________________ Reaction ________________

Chemical Examination

Albumin ________ neg. ______________ Sugar ________________ neg. ______

Acetone ________ neg. ______________ Bile ________________ neg. ______

Blood ________ neg. ______________

Microscopic Examination

Casts ____

W.B.C. per H.P.F. __________________ R.B.C. per HPF ________________

Epithelial Cells __________________ Crystals ________________

Other ____

Match Column I with Column II

Column I		Column II
____	1. organized sediment	a. advanced nephritis
____	2. pus corpuscles	b. crystals in acid urine
____	3. cylindroids	c. frequently appear in urine of females
____	4. calcium oxalate crystals	d. degeneration of the renal epithelium
____	5. fatty casts	e. suppuration in urinary tract
____	6. epithelial cells	f. formed in renal tubules
____	7. crenated	g. cells and other formed elements
____	8. tube casts	h. crystals in alkaline urine
____	9. waxy casts	i. notched or indented edge, shrunken
____	10. ammonium biurate	j. resemble hyaline casts

II. HEMATOLOGY

Objectives

Develop appreciation of the importance of Hematology in diagnosis

To learn how to charge and properly focus the counting chamber

A. Erythrocyte Count

The red bone marrow forms red blood cells. These cells are called erythrocytes. Red cells are non-nucleated, biconcave disks. They carry oxygen to the cells and a chemical in them called hemoglobin, unites with oxygen to form oxyhemoglobin. Besides transporting oxygen they also carry carbon dioxide and contain an antibody called the Rh factor. When the body does not produce sufficient red cells anemia results. If too many red cells are produced polycythemia results.

1. Method: The method used for determining the number of red cells makes use of the hemacytometer. This consists of a counting chamber, cover glass and pipet for diluting the blood.
   a. The reagent used for diluting the blood is Hayem's solution. This may be purchased prepared or mixed as follows:

Sodium chloride	1.0 gm.
Sodium sulfate	5.0 gm.
Mercuric chloride	0.5 gm.
Distilled water	200 cc.

   b. preparation of slide:

(1) equipment:

disposable lancet	R.B.C. diluting pipet
alcohol	R.B.C. diluting fluid
cotton sponges	counting chamber
microscope	mechanical counter

(2) Obtain a drop of blood following the finger puncture technique

(3) Insert the pipet into the drop of blood
Do not touch the pipet to the donors finger

(4) Draw blood to 0.5 mark on pipet. Insert the pipet into the diluting fluid and draw up to the 101 mark. This makes a dilution of 1:200. Rotate the pipet between your thumb and forefinger to avoid blood clots while it is filling.

(5) Mix the cells through the diluting fluid by shaking, in a figure eight motion, for three minutes.

(6) Charge the counting chamber by placing the pipet at an angle to the edge of the cover glass. Release pressure slightly so that the dilution completely fills the space between the chamber and the cover glass.

(7) Allow the cells to settle for several minutes.

(8) Place the chamber on the microscope stage and using the low power objective, check for uniformity of distribution and any extraneous material.

(9) Switch to high power and adjust for lighting and focus.

c. Calculation

(1) Count all the cells present in the area squares marked a, b, c, d, and e (see figure). In counting the cells in the squares count only those cells which touch the line on the left and top of the squares. The difference between any two squares should not be more than fifteen. If the difference is greater repeat the test from the beginning. Total all cells counted in the squares.

(2) Counting the cells at the beginning may best be accomplished by counting from left to right across each row of four small blocks. After much practice a sweeping motion of left to right and then right to left on the next row is more easily employed.

(3) A short method of calculation is available which is both adequate and quick. After totaling the number of cells present in all eighty squares multiply the total by 10,000 (or add four zeros to the total).

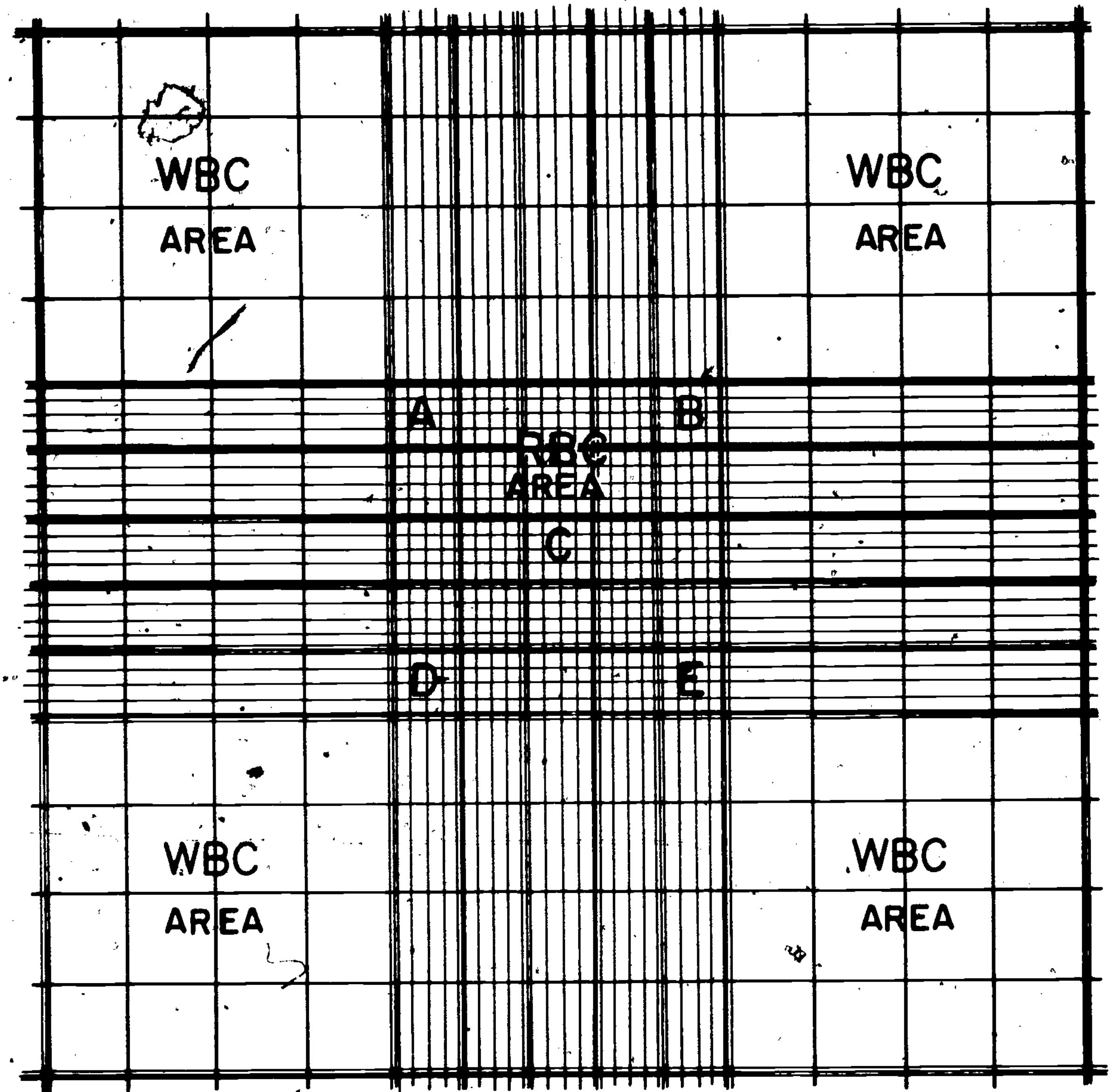
Example: total count is 490 with four zeros added becomes 4,900,000

d. Normal values

Men	4.5 to 6.0 million per cu. mm.
Women	4.0 to 5.5 million per cu. mm.
Infants	5.5 to 7.0 million per cu. mm.

e. Sources of error

Failure to hit the 0.5 mark when filling the pipet with blood
Allowing the blood to clot
Inaccurate dilution
Contamination of the diluting fluid with red cells
Overfilling or scanty filling of the chamber
Not shaking or incorrect shaking of the pipet after diluting
Poor sample of blood
Error in counting the cells, usually results in low count


WBC
AREA
WBC
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AREA

Review

1. Red cells are formed in the ______________________________

2. Red blood cells transport oxygen and ______________________________

3. The normal erythrocyte count for women is __________ and for men __________

4. Inability of the long bones to produce sufficient red blood cells results in __________

5. Polycythemia is the disease in which red cells are produced ______________________

6. When counting the cells there should not be a difference greater than __________ between any two squares.

B. Leukocyte Count

Objectives

Become adept at performing W.B.C.

Develop appreciation of importance of W.B.C. in diagnosis

Leukocytes are manufactured by the bone marrow, spleen and lymph nodes. The leukocytes are the cheif defense against disease. They actually engulf and digest the invading microorganisms. This process is called phagocytosis and the cells are called phagocytes.

The purpose of the leukocyte count is to determine the presence of infection in the body. An abnormally high white count, leukocytosis, will verify suspected infections. Leukemia will increase the count to approximately 25,000 to 1,000,000 or more. Leukopenia is an abnormally low count and could be the result of poor response to infection. The leukocyte count is a good indication of a person's nutritional habits since well nourished individuals will have a count in the upper half of the normal range.

1. Method of leukocyte count

   The method of performing a leukocyte count is basically the same as for a erythocyte count, the difference being the reagent and the pipet used. In view of this it will not be necessary to repeat the entire procedure here but just to note the differences.

   a. Pipet – the W.B.C. diluting pipet differs in that it will have a smaller bulb, which contains a small white or colorless bead and results in less dilution of the blood. The fifth line is marked 0.5, the tenth line 1.0 and above the bulb 11. This results in a dilution factor of 20. The most popular diluting fluid is Turck's solution which is prepared as follows:

Glacial acetic acid	3 ml.
Distilled water	97 ml.
1% gentian violet	1 gtt.

b. **Procedure**

Same as for erythrocyte count but using the appropriate pipet and diluting solution.

c. Calculation

W.B.C. areas are marked in the figure and located in the four corners of the chamber. The difference between the number of cells in each square should not exceed 10. Only those cells touching the top and left borders as well as those within the square are counted.

Multiply the total number of cells in all four squares by 50.
Example: count is 150 by 50 = 7,500

or

150 to which add two zeros and then divide by 2

d. Normal values

The normal range in adults is from 5,000 to 10,000 per cubic millimeter of blood. The average normal is 7,500. The normal range for children is considered to be from 8,000 to 11,000.

e. Sources of error

Impurities in the diluting fluid
Improper cleaning of the pipet or chamber
Other sources of error are similar to those listed for erythrocyte counts.

Review

1. Leucocytes are manufactured by the __________ __________ and __________
2. The main purpose of the leukocyte count is __________
3. An abnormally high white count is called __________
4. One cause of an abnormally low white count is __________
5. A leukocyte count from 25,000 to 100,000 usually indicates __________
6. An indication of a person's __________ habits may be learned from a leukocyte count.
7. How does the W.B.C. diluting pipet differ from the R.B.C. pipet?
8. Normal W.B.C. for adults is from __________ to __________
9. __________ is the diluting solution used for W.B.C.
10. When calculating the number of cells present in a W.B.C. the difference between any two squares should not exceed __________

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C. Differential Count Smears

Objectives

Learn the importance of differential count smears

Demonstrate ability to prepare smears

Acquire ability to classify and report the types of leukocytes

1. Method

There are two general categories of leukocytes which may be classified microscopically. These are granulocytes and nongranulocytes (lymphocytes). The subdivisions and descriptions of these will follow. The differential count is vitally important in a complete blood count as it may well serve to provide the correct diagnosis to the physician. It may also indicate if radiotherapy or chemotherapy are producing harmful effects. Only those smears prepared with the greatest exactness and care will be totally reliable. Never attempt to do a count from a less than perfect smear. For this reason it is suggested that two smears always be prepared to provide a spare should one smear prove defective for diagnostic purposes.

a. Preparation

There are several precautions to be observed to insure the best possible smears.

The slides must be perfectly clean

The drop of blood must be perfectly clean.

The drop of blood must not be too large

Complete the work quickly so coagulation does not occur

Never use oxalated blood for smears

b. Staining

Materials

finger puncture equipment	distilled water or
2 glass slides	buffer solution
staining rack	medicine droppers
Wright's stain	immersion objective
	immersion oil

Procedure

(1) Puncture finger as for a count

(2) Touch the drop of blood to the slide – do not touch the finger

(3) Place the slide on the table and hold it at the far end with your thumb and middle finger of left hand. Place another slide in front of the drop of blood at an angle of approximately 45°. The smaller the angle the thinner the smear.

(4) Pull the spreader slide back to the drop of blood. Hold it in place until the drop of blood has flowed across almost the entire width of the spreader.

(5) Using a firm steady motion push the spreader across the surface of the slide until the blood is smeared across most – but not all – of the slide

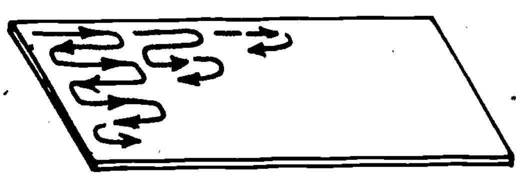
(6) Allow the slide to dry and then write the patient's name with a lead pencil through the thick end of the smear.
(7) Place the slide, film side up, on the staining bridge over the staining pan and use a medicine dropper to apply Wright's stain to cover generously. (approximately 16–20 drops)
(8) Allow the stain to remain in place for 1–3 minutes.
(9) At the end of 1–3 minutes add the buffer solution drop by drop until a slightly greenish film covers the surface. Be careful not to let the stain and buffer solution overflow. Buffer should be added in amounts equal to the stain.
(10) Blow gently across the surface of the slide to mix the stain and buffer. Let the slide sit for 2–6 minutes after the appearance of a metallic-like scum. At the end of this time float the stain and buffer off by using a gentle flow of distilled water. Keep the slide in place on the rack during this process. Continue rinsing until all traces of the excess stain have vanished.
(11) Drain by tilting the slide and then air dry by tilting or waving the slide gently.
(12) When dry place a drop of immersion oil on the slide near the thin end or the middle. Place the slide on the stage under the oil immersion objective.

c. Classification

Classify 100 leukocytes. Be careful to follow a pattern in examination. The total number of each kind classified represents the percentage of each.

See illustration

Much practice is needed to perform the test speedily and accurately. Do not be discouraged if you encounter some difficulty on your first few attempts. Practice should resolve your difficulties.



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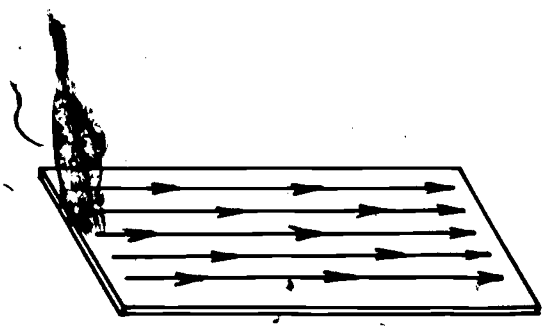

B

PATTERNS FOR COUNTING CELLS

d. Results may be recorded as follows:

Example

I. Granulocytes	Normal values/100 cell count
A. Neutrophils	
1. Segmented polys	60–70%
2. Stabs or bands	2–5 %
B. Eosinophils	1–3%
C. Basophils	0–1%
II. Nongranulocytes	
A. Lymphocytes	20–35%
B. Monocytes	2–6 %

D. Main Types of Leukocytes

1. Granulocytes

   a. Segments neutrophils – these are commonly called sets or polys (for polymorphonuclear leukocytes). They are round or oval cells in which the cytoplasm is lavender or a pink color with lavender or pinkish granules. The nucleus is in segments having anywhere from two to twelve segments. All cells with five or more segments must be reported.

   b. Stabs or bands – these neutrophils are commonly called stabs, bands or rods. They are round or oval with a cytoplasm similar to that of the segs. Their nucleus is one continous piece commonly shaped like the letters c, s, n, or u. This nucleus is dark purple or lavender and usually occupies about one fourth of the cell.

   c. Eosinophil – these are commonly called eos. They have coarse, round or oval bright pink or red granules which cover the cytoplasm. The cytoplasm itself has a light blue tinge. The segmented nucleus, usually having two lobes. They stain a deep lavender to a light blue.

   d. Basophils – these are commonly called basos. The cytoplasm is covered with coarse, dark purple granules that may also be found on the nucleus. The nucleus takes a lavender or purple stain and the cytoplasm a neutral or bluish tinted stain.

2. Nongranulocytes

   a. Lymphocytes – these are commonly called lymphs. The purple or lavender nucleus of this cell may be so large as to almost totally obscure the negligible blue cytoplasm. The mature lymph is very small and resembles the red cell in size.

   b. Monocytes – these cells are called monos. These are the most delicate as well as the largest of the white cells. The cytoplasm is a smoky blue-grey sprinked with a pink dust. They are somewhat irregular in shape. The nucleus, which is lobulated or horseshoe shaped, stains a lavender color.

Review

1. What is a differential count smear?
2. Name the solution used to fix and stain the slide.
3. List the equipment needed for a differential count smear.
4. List the leukocytes under the following headings:
   a. granulocytes
   b. nongranulocytes
5. What is the importance of the differential count to the physician?
6. How many cells must be observed in the differential count smear?
7. What microscope objective is used in viewing the smear?

HEMOGLOBIN DETERMINATION

Objectives

Develop the necessary skills to perform hemoglobin determinations Acquire skill in reading and reporting results

Determine the best method available and perform tests accordingly

E. Hemoglobin Estimation

Hemoglobin is found in the red cells and gives blood its red color. Its basic components are an iron constituent, hematin, and globin, a protein constituent. The determination of hemoglobin is important in the diagnosis of anemia. Hemoglobin is increased in polycythemia and hemoconcentration and decreased in anemia and hemorrhage. A hemoglobin estimation is considerably more useful than an erythrocyte count as it detects the existence and degree of anemias and indicates the effects of treatment on anemic conditions.

1. Methods are classified as direct or indirect. The direct method relies on matching the color of a specimen to a standard. This method is unreliable since it depends upon a person's ability to differentiate shade variations and color. If, however, the matching is done photoelectrically the reliability is greatly increased.

Materials

Tallquist Method (direct method)

Finger puncture equipment

Tallquist hemoglobin scale – this includes a book of small sheets of paper and color scale showing values ranging from 10 to 100%.

Procedure

(1) Perform a routine finger puncture
(2) Blot a drop of blood with the absorbent paper
(3) Allow the paper to dry until the sheen has disappeared
(4) Match the paper against the color standard. Use the lightest possible background and sunlight if possible.
(5) Note the figure of the color closest to the color of the test spot.
(6) Report the results in percentages
Example: 80% (Tallquist)

b. Hemometer

Materials

finger puncture equipment

Hemometer

Procedure

(1) A drop of blood is placed on the provided slide. This is mixed thoroughly until changed into a clear red – 45 seconds.
(2) The chamber is then covered and inserted into the instrument
(3) The specimen is then viewed through the eye piece by pressing the illuminating button. The resulting shade is matched with the standard by moving a slide on the side of the hemometer until both shades of green match.

(4) When the match is completed the reading is taken from the side of the chamber where the slide rests

(5) Report as: 14.5 per 100 ml.

(a) Sahli Method (Indirect Method)

Materials

finger puncture equipment

Sahli-Hellige hemometer or hemoglobinometer with calibrated tube, 17 gm.

Shali hemoglobin pipet

0.1 N hydrochloric acid

distilled water

glass stirring rod and dropper for water

2. Fill the calibrated tube with 0.1 N hydrochloric acid to the 2 gm. mark
3. Draw blood to the 20 cu. mm. mark on the pipet
4. Put the pipet carefully into the calibrated tube so that the tip of the pipet is at the bottom of the HCL. Expel the blood slowly being careful not to form bubbles.
5. After expelling the blood, rinse the pipet by slowly drawing the solution into the pipet and expelling it several times.
6. Stir the solution with a glass stirring rod. Allow this to stand for ten minutes so that the hemoglobin is turned into acid hematin.
7. Place the tube into the hemoglobinometer. Hold this up to daylight or fluorescent lighting and add distilled water one drop at a time. Stir after each addition.
8. Continue this until the color of the solution matches the color of the brown glass plates of the hemometer.
   When checking the color do not remove the glass rod completely. Merely lift the rod above the solution so no solution is lost causing an inaccurate reading to be made.
9. If you are uncertain if the solution will take another drop, note carefully when this occurs and then add a drop. If this is too dilute then use the previous reading.
10. To read, remove the tube from the instrument, lift the rod above the fluid level in the tube and record the point at which the meniscus of the solution is tangent with the calibration scale.
11. Record the reading. The reading corresponds to gm. and percent of hemoglobin. The 17 gm. = 100% tube must be used.

b. Color Index

Color index means the amount of hemoglobin in each erythrocyte as compared to the normal amount. The color index is determined by multiplying the first two numbers of the erythrocyte count by two and dividing the product into the percentage of hemoglobin.

Example: Erythrocyte count = 5,000,000

$$\text{Hemoglobin} = 100\%$$
$$\frac{100\%}{50 \times 2} = 1.0$$

c. Normal values

Normal value for men is from 14 to 18 gm. per 100 cc. of blood (90 to 110%). The normal value for women is 12 to 16 gm. per 100 cc. of blood (80 to 100%). Determinations for children decline from birth when they are at a high of 20 to 22 gm. and decrease gradually to about 12 to 14 gm. at age 12.

d. Sources of error

Direct Method – using a drop of blood that is mixed with cell fluid
Inability to differentiate shades of red
Indirect Method – drawing blood too far into pipet or not far enough, damaged pipets or dirty equipment
Failure to measure the HCL accurately, and not lifting the stirring rod above the fluid level before making the reading and before matching.

Review

1. Give one advantage and one disadvantage of using the Tallquist Method of hemoglobin determination.
2. Do a hemoglobin on a volunteer using the Tallquist and Sahli methods. Compare your results for accuracy. Use the same donor for both tests.
3. Why is the hemoglobin determination more valuable to the physician than the erythrocyte count?
4. In what conditions will the hemoglobin be elevated?
5. Give the normal values of hemoglobin.
6. What does the color index indicate?
7. How is the color index arrived at?
8. In the Sahli Method, why is a ten minute wait necessary after adding the blood to the HCL?
9. Why is the indirect method more accurate than the direct method?
10. What two terms are used in reporting hemoglobin?

F. Erythrocyte Sedimentation Rate

The Erythrocyte Sedimentation Rate is the distance that the red corpuscles have fallen after a given interval of time. The rate is valuable in following the course of certain inflammatory diseases such as rheumatic fever and tuberculosis. There is an increase in the E.S.R. in rheumatoid arthritis and localized acute infection. It is also useful in diagnosing and following the course of myocardial infarction. The E.S.R. may be taken as a non-specific index of the presence and intensity of organic disease.

Objectives
To understand the value of the Erythrocyte Sedimentation Rate
Acquire proficiency in performing the test
Accurately report and record the test results

a. Method
The method commonly used is the Wintrobe method – Wintrobe E.S.R.
Venous blood is used for this test and care must be exercised that the cells are not damaged during aspiration by use of a too small needle.

Materials
sterile 5 cc. syringe
#18 and #19 needles recommended
test tube – dried double oxalate mixture
Wintrobe hematocrit tube and rack
Capillary pipet
Centrifuge

Procedure
1. Draw 5 cc. of blood into the syringe and place it in an oxalated tube.
2. Cork the tube and invert it at least ten times.
3. Withdraw blood from the tube using a pipet and bulb or a pipet and syringe.
4. Place the tip of the pipet at the bottom of the tube and begin expelling the blood while raising the pipet.
   Keep the tip of the pipet just below the surface of the blood. Be careful not to form bubbles. Fill the tube to the zero mark of the scale.
5. Place the tube in the rack. Be certain the tube is absolutely vertical and do not disturb it for one hour.
6. At the end of the hour, read the level of the junction between the plasma and the cells. Use the appropriate calibration on the tube for your reading. The calibration is located on the left reading from 10 at the bottom to 0 at the top.
7. Place the tube in the centrifuge for 30 to 40 minutes at 3,000 rpm and read the volume of packed cells. Refer to a chart for the corrected rate. Record the rate in mm./1 hour.

b. Normal values

The normal sed rate, using the Wintrobe method, is 4 mm. for men with the maximal normal range being 0 to 9 mm.; for women the normal is 10 mm. with the range being 0 to 20 mm.

c. Source of Errors

Blood should be collected between 10 A.M. and 4 P.M. as the time of collection will influence the rate.

Conduct the test as soon as possible after collecting the sample. Temperature is an important factor. The room temperature should be between 22 – 27°C.

If blood has been chilled allow it to return to room temperature.

Anticoagulants, except heparin, will influence the rate. If these drugs are used as prescribed they may not influence the test greatly.

Test tubes should not be smaller than 2 mm.

G. Hematocrit

Method

Hematocrit separates the solid elements of the blood from the plasma and measures the volume of packed red cells. The method is basically the same as for the E.S.R. and may be done separately or after the completion of the E.S.R.

Materials

The same as for E.S.R.

1. Procedure

   Follow the procedure for E.S.R.

   After centrifuging and reading the sed rate on the left side of the Wintrobe tube the hematocrit can be read on the right side. This side of the tube reads from zero at the bottom to ten at the top. The hematocrit is determined by reading from the bottom of the tube. Do not include the layer of white cells and platelets on top of the red cells in the reading. Only the volume of the packed red cells is read.

2. Normal Values: for females is 42 cc. or % and for males 47 cc. or %.

Review

1. The normal range in E.S.R. for men is from __________ to __________ and for women from __________ to __________
2. List three diseases in which knowing the E.S.R. would be helpful.
3. What is an anticoagulant?
4. Why are #18 and #19 needles recommended for drawing blood for the E.S.R.?
5. The common method in use for determining the E.S.R. is the __________
6. Why is a tube containing oxalate used?
7. First determination of E.S.R. is made by reading the level of the junction between the ________ and the __________
8. Hematocrit separates the __________ elements of the blood from the plasma.
9. An increased hematocrit may indicate __________
10. Using a volunteer do an E.S.R. and hematocrit and report on the form below:

Name __________________ Date __________

Age __________ Sex __________

Sed Rate __________	normal	0.9 mm. Male
	normal	0 – 20 mm Female
Hematocrit __________	normal	47% Male
	normal	42% Female

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H. Bleeding Time and Coagulation Time

1. Diagnostic Importance – Bleeding Time
Bleeding time is a test to determine the time needed for bleeding to stop after a small puncture wound.

   a. Normal bleeding time is from 1 to 3 minutes although it may be as prolonged as 8 minutes. When bleeding continues past ten minutes it is considered to be seriously prolonged.

Materials
finger puncture equipment
filter paper or coarse gauze
watch with second hand

   b. Procedure

      (1) Make a finger puncture sufficiently deep to produce a large drop of blood. Do not squeeze the finger.
      (2) Note when the first drop of blood appears.
      (3) Using the filter paper, or gauze, blot off the first drop of blood and each subsequent drop at thirty-second intervals
      (4) The time between the appearance of the first drop of blood and the last represents the bleeding time.

2. Diagnostic Importance – Coagulation Time
Coagulation means the time it takes for the blood to clot. It is important in the diagnosing of hemorrhagic diseases and before surgery. Coagulation is seriously impaired in hemophilia when the clotting time may be from one to several hours. It may also be prolonged because of decreased thromboplastin, prothrombin, fibrinogen or because of the ingestion of anticoagulants.

   a. Normal values –
   When using a skin puncture for the test, normal clotting will occur in 4½ minutes with the range being 2 to 6 minutes. Clotting may be more rapid after meals.

Materials
finger puncture equipment
capillary tubes, 1 to 1.5 mm. diameter
watch with second hand

b. Procedure

(1) Make a puncture sufficiently deep to produce a free flow of blood
(2) Wipe away the first drop of blood
(3) Hold the tip of capillary tube so that it touches the blood and fills. Allow the blood to rise about 2/3 of the way up the tube.
(4) Wait about 1½ minutes before breaking off the first segment. Continue breaking off segments at 30-second intervals. This must be done gently.
(5) Coagulation is considered to have occurred when fibrin threads appear between broken segments. Coagulation time is the time elapsed between the appearance of blood and the appearance of the fibrin threads.

Review

1. The normal bleeding time is from ________ to ____________
2. Bleeding past __________ means it is abnormally prolonged.
3. The clotting of blood is called___________
4. Coagulation time is important in the diagnosing of __________ diseases.
5. Coagulation is prolonged in _______________
6. Normal coagulation time is from __________ to ____________
7. Coagulation is considered to have occurred when ______________ appear between the broken segments of the capillary tube.

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I. Blood Typing and Rh Factor

Objectives
Understand the importance of accuracy in Blood Typing
Acquire skill in the performance of both typing and Rh Factors

1. Blood typing and Rh Factor

   a. Blood typing is the classification of human blood into four basic blood groups. The group is determined by the presence or absence of agglutinogens and agglutinins. Type O, the commonest of the groups, has no agglutinogens but contains both Anti-A and Anti-B agglutinins. Type O represents 45% of the population. Type A has A agglutinogen and Anti-B agglutinin. This type of blood accounts for about 40% of the population. Type B has B agglutinogen and Anti-A agglutinins. Type B comprises about 10% of the population. Type AB has both Anti-A and Anti-B agglutinogen and no agglutinins. This accounts for the remaining 5% of the groups.

   b. Normal values and Compatibility
   Type O is called the universal donor as adding this type to any of the others will rarely result in any serious reactions. Since persons belonging to the AB type group possess no agglutinins they are designated as the universal recipient.

   Materials

Microscope	Clean toothpick
Slide	Anti-A serum
Marking pencil	Anti-B serum

   c. Procedure
      1. Use a thoroughly clean and dry microscope slide.
      2. Using a china marking pencil, mark the slides as indicated in the figure.
      3. Place a drop of Anti-A serum in the circle marked A.
      4. Place a drop of Anti-B serum in the circle marked B.
      5. Proceed for a routine finger puncture and use the second drop of blood for test.
      6. Using a clean toothpick, obtain a small drop of blood from the puncture and mix it well with the drop of Anti-A serum.
      7. Do the same for the Anti-B.
      8. Rock the slide back and forth gently for 2-3 minutes. Be careful to keep the mixtures within the range of the rings drawn.
      9. Compare the results with the illustrations for identification. If no agglutination has occurred the mixtures will remain uniform in appearance. If a granular appearance results agglutination has occurred.

2. Rh factor derives its name from the rhesus monkey used in the experimentation to study immunologic relationships. The Rh factor is the term applied to one or more agglutinogens found in the red blood cells. If the blood contains this type of agglutinogen it is said to be Rh positive. Approximately 85% of the population have an Rh positive factor and the remaining 15% are Rh negative.

a. Importance of the Rh Factor

If the Rh factor enters the Rh negative blood during transfusion the negative body will produce an antibody against it. This reaction is similar to that which occurs upon introduction of foreign protein and disease products. The two instances when the Rh factor is of vital importance are in transfusion and pregnancy. During pregnancy, should the Rh negative mother bear an Rh positive child some of the antibodies from the mother may pass from her blood to the child. This could result in fetal death, erythroblastosis fetalis (hemolytic disease of the newborn). This does not usually occur with the first child but will with subsequent births. In recent times an immuno globulin has been developed which will suppress the antigen stimulus during the post partum period. This is called Rhogam and is administered within 72 hours following delivery.

Materials
microscope slide
china marking pencil
Anti-Rh serum

b. Procedure
(1) Use a clean, dry microscope slide
(2) With a china marking pencil, draw a single circle in the middle of the slide
(3) Place a drop of the Anti-Rh serum in the circle
(4) Follow the finger puncture technique for a blood specimen
(5) Turn the finger over and hold it above the Anti-Rh serum so that the blood drops onto the slide
(6) Mix the blood and anti-serum thoroughly
(7) Rock the slide gently for two minutes
If the slide is heated to about 37 °C. the reaction will be accelerated
(8) Examine the results

Rh+	agglutination
Rh-	no agglutination

Review

1. Using a medical dictionary define the following:

agglutination	antibody
agglutinin	erythroblastosis fetalis
agglutinogen	immunologic
serum	universal donor
	universal recipient

2. Fill in the results obtained from the following blood test:

    a. no clumping on either side Type__________

    b. clumping on the Anti-A side Type__________

    c. clumping on the Anti-B side Type__________

    d. clumping on both sides Type__________

3. What is the origin of the name Rh?
4. What percentage of the population has the Rh factor?
5. The four main blood groups are _____, _____, ______, and ______
6. Using a blood sample from yourself, perform both a typing and Rh test.
7. The two instances when the Rh factor are of vital importance are ________________
8. How can the production of Rh antibodies be prevented postpartum?
9. Type________ is called the universal donor and type AB is designated universal ____

J. Blood Chemistry

Objectives

Acquire skill in the execution of the various test kits available for use in the office

A. Method – Blood Glucose

Materials

finger puncture equipment
reagent strip
small amount of water

Procedure

1. Apply one drop of whole blood to the test strip.
2. Wait one minute and wash off with water.
3. Compare the resultant color with the color chart provided.
4. The intensity of blue measures glucose as 45 mg./100 Ml. to 250 mg./100 ml.

B. Method – Infectious Mononucleosis

Materials

Mono-test reagent
Negative and positive controls
capillary tubes with rubber bulbs
glass slide

Procedure

1. Fill the capillary with serum to the mark and place it in the middle section of the slide.
2. Place one drop of negative control in the right section of the slide and one drop of positive control in the left section.
3. Place one drop of reagent in each section.
4. Mix separately using a clean stirrer for each section.
5. Rock gently for two minutes and observe for agglutination. Use a high intensity lamp and observe against a black background.
6. The test is reported as negative or positive.

C. Method – Sickle Cell Anemia

Materials

finger puncture equipment
Bugger
test tubes 12 x 75 mm.
lined card
Reagent powder

Procedure

1. Mix the buffer and the reagent powder and agitate until the powder has been dissolved
2. Add 20 $_{u}$l of blood to 2.0 ml of reagent in the test tube. Incubate for five minutes at room temperature and observe for turbidity by holding the test tube one inch in front of the lined card.

   Positive – lines on card cannot be seen through test tube.

   Negatige – lines on card can be seen through test solution.

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III. Bacteriology

Objectives:

To acquire facility in identification of the forms of bacteria

Knowledge of the various test media for growing cultures

Ability to prepare smears and stains as needed for identification purposes

A. Bacteria are single-celled microscopic plants rich in nucleo-protein. About 1500 species of them are known and approximately 40 or 50 of them are injurious to man. Under ideal conditions bacteria will reproduce by a process called binary fission. This is asexual cell reproduction by means of cells dividing and then each segment reproducing in a like manner. It is because of their rapid reproduction that bacteria are so potentially dangerous to man.

   Bacteria are divided into three major groups which will be considered here.

   1. coccus – spherical bacteria
      a. staphylococci
      b. diplococci
      c. streptococci
   2. bacillus – rod shaped
   3. spirillum – curved in a spiral manner

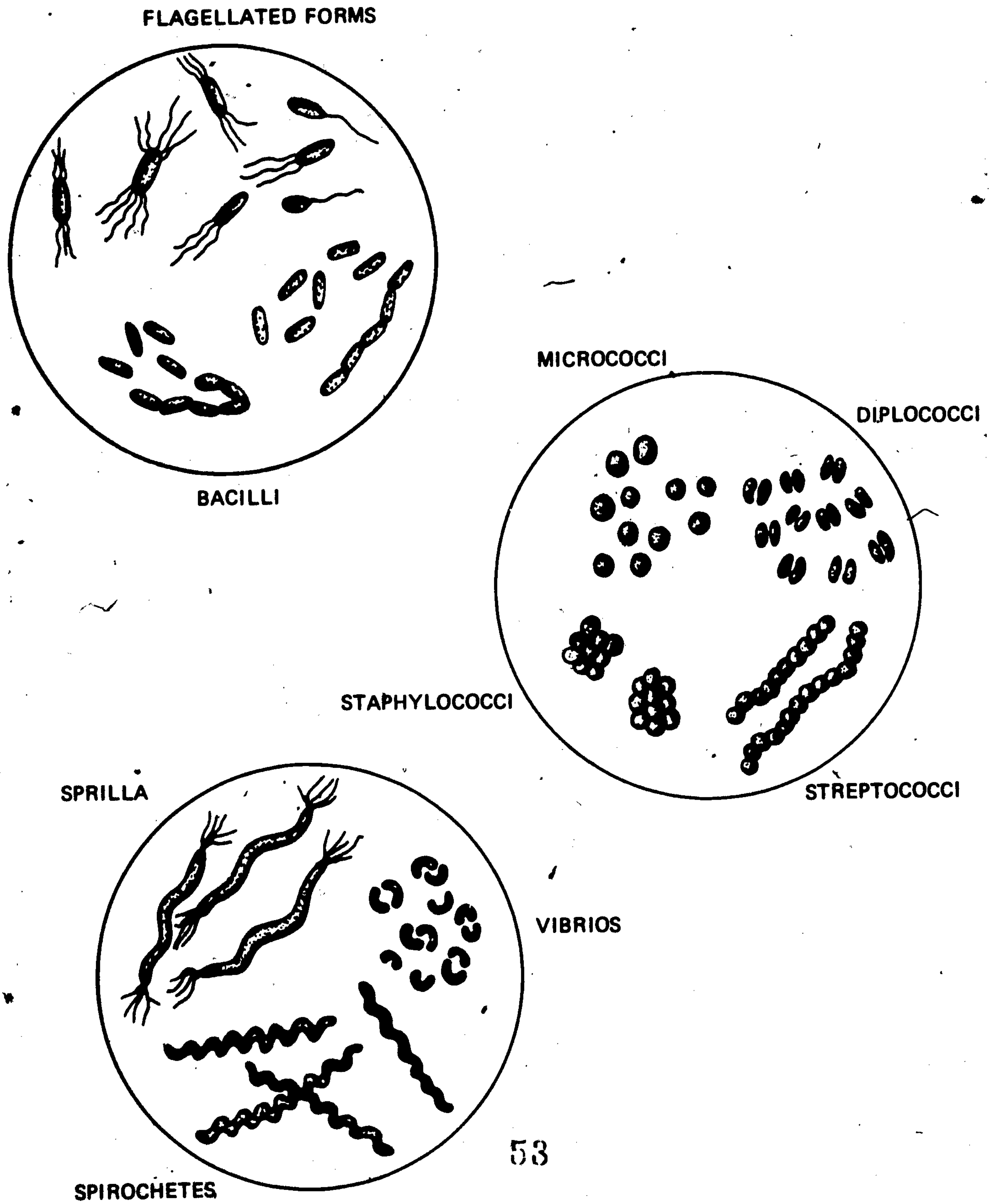
   1. Coccus – several of these are capable of producing disease and therefore important to this study. The staphylococcus is found virtually everywhere in nature. The pyrogenic, pus producing staphylococci are usually implicated in pimples, boils, osteomyelitis, etc. Diplococci, those found in pairs, are responsible for such diseases as pneumonia and gonorrhea. Streptococci; forming chains of cells, cause strep throat, scarlet fever, septicemia and rheumatic heart disease.

   2. Bacillus – this form of bacteria is the most common.
      It is the cause of typhoid fever, tuberculosis and bacillary dysentery.

   3. Spirillum – this is the least common form of bacteria. It is responsible for cholera and syphillis.

B. Preparation of Culture

   Culture methods are employed for isolation, identification and preservation. The medical assistant will be concerned principally with isolation and identification. A culture contains living organisms. The solid media commonly used for cultures is nutrient agar. Solid media is usually preferred as bacteria will grow in colonies made visible under the low power objective of the microscope. If liquid media is used the bacteria pile up in mixed masses and are difficult to identify.


FLAGELLATED FORMS
BACILLI
MICROCOCCI
DIPLOCOCCI
STAPHYLOCOCCI
STREPTOCOCCI
SPRILLA
VIBRIOS
SPIROCHETES

1. a. One dish – streaking an agar plate

This method is frequently used to obtain a growth of known pure cultures when it is not necessary to obtain well isolated colonies.

Materials

Inoculating needle
one petri dish – sterile with culture media
material to be cultured
bunsen burner
incubator

Procedure

(1) With a sterile inoculating loop, place two loopfuls of the material to be cultured near the edge of the plate.
(2) Sterilize the loop by heating it to a red heat and allow it to cool. This will prevent scattering.
(3) Apply the loop to the material placed on the plate and streak gently without breaking the surface. Sterilize the loop before and after each streaking. Using this method, part of the plate will be heavily streaked and part lightly. This will provide for isolated colonies.
(4) Put the plate into the incubator at 37°C. Place it in an inverted position, media side up.
(5) Examine the plate after 24 hours for growth. Various cultures will need from 24 hours to several weeks.
(6) After incubation, some of the colonies will be fished for pure cultures and others for staining. If necessary use a straight wire for fishing if the colonies are close together.

Identify single colonies by observing them with a single hand lens or under the low power objective of the microscope. Ring and number the colonies on the bottom of the petri dish. Using a sterilized, straight inoculating wire touch the tip of the needle to the colony. Prepare smear etc. as needed.

b. Three Dish Method

Materials

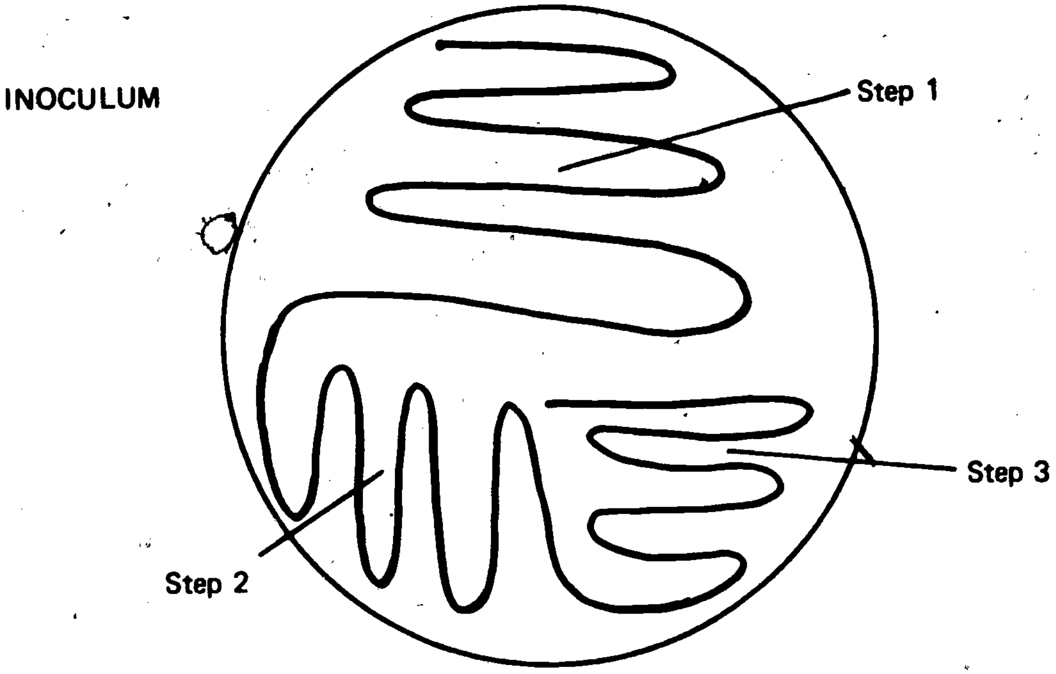
Three petri dishes with appropriate sterilized media; other materials as for one dish method

Procedure

(1) Flame an inoculating loop and obtain a loop of the material.
(2) Streak the three dishes in succession without obtaining any additional material.

Using this method will result in bacteria on one or more of the plates developing into distinct colonies.

2. Precautions should be observed as to sterilization, cleanliness, etc. since it will be possible for contamination to occur.



3. Agar Slant Method

Material

inoculating loop	Bunsen burner
nonabsorbent cotton	pure culture tube

Procedure

a. Label the culture tubes and loosen the plugs.
b. Flame the loop.
c. Remove the plugs from the tubes and flame them.
d. Flame the lips of the tubes.
e. Place a small amount of material from culture tube into the fresh agar medium.
f. Flame the lips of the tubes again and replace the plugs.
g. Flame the loop.
h. Incubate the tubes.

4. Examine after 24 hours. Once again, the incubation period may last from 24 hours to several weeks.

C. Smear from Streaked Plate

1. a. Before bacteria can be viewed microscopically for identification they must be processed to prepare them.

   Procedure

   (1) Begin by cleaning a glass slide and marking it with a circle in the middle.
   (2) With a flamed loop, place a drop of clean water in the circle.
   (3) Mix an adequate amount of material to be stained, with the drop of water.*
   (4) When evenly spread over the area allow the slide to air dry.
   (5) After drying, pass the slide through the flame of the bunsen burner two or three times to fix it. Place on a rack for staining.

   * If the material is liquid omit the drop of water.

   b. Smear from an Agar Slant
   (1) Remove the cotton plug. Flame the mouth of the tube
   (2) Flame the loop, remove the specimen from the tube and place it on a clean glass slide.
   If the specimen is in the liquid state, it will not be necessary to add a drop of water. If the specimen is solid matter add one drop of water to the slide and mix well.
   (3) Flame mouth of test tube and replace the plug.
   (4) Return the tube to incubator.
   (5) After mixing and spreading, the slide is allowed to air dry.
   (6) After drying, fix by passing the slide through the flame of the bunsen burner two or three times.

D. Staining of slides

Gram stain – stain reaction will be either negative or positive.

1. a. Cover slide with crystal or gentian violet stain one minute.
   b. Add five drops of 5% sodium bicarbonate solution to the stain on the slide.
   c. Pour this off and add Gram's iodine solution for one-half minute.
   d. Quickly decolorize with acetone.
   e. Wash with water.
   f. Dry and mount a cover slip.

2. Gram positive bacteria are those that hold the violet stain.
   Gram negative are those that take the color of the counter stain such as the red of the safranin.

3. Acid Fast Stains – this is used for the staining of the tubercle bacilli and certain other related germs.

   Procedure
   a. Apply carbolfuchsin to cover the smear and heat it until steaming hot. Everying on the slide will appear intensely red.
   b. Wash the stain off and add acid alcohol.
   c. Stain with methylene blue.
   d. The slide should next be thoroughly dried.
   e. Examine under the oil immersion objective.

Review

1. The three major groups of bacteria are ________ , ________ and ________
2. Of all species known approximately ________ are injurious to man.
3. Bacteria reproduce by a process called ________
4. Describe briefly the shape of the three major groups of bacteria.
5. The ________ staphylococcus is responsible for boils, osteomyelitis, etc.
6. The commonest form of bacteria is ________
7. Syphillis is caused by the ________ bacteria.
8. Cultures are employed for ________ , ________ and ________
9. The two types of culture media are ________ and ________
10. Give one advantage to using nutrient agar as a media.
11. What method is employed in growing cultures if it is not necessary to obtain well isolated colonies?
12. The method best employed for producing distinct colonies of bacteria is ________
13. Cultures may be grown on solid media in test tubes called ________
14. Explain the process for preparing a smear from a streaked plate culture.
15. When preparing a smear from a liquid culture it is not necessary to add a ________
16. Gram positive bacteria can be identified by their ability to hold the ________ stain.
17. Gram negative bacteria will be ________ the color of the counter stain.
18. If tubercle bacilli are present the stain used is the ________
19. The two stains used to prepare an acid fast stain are ________ and ________
20. Stains are examined under the ________ objective of the microscope.

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